Journal of Pioneering Medical Sciences
Received: June 26, 2025 | Accepted: October 11, 2025 | Published: December 05, 2025
Volume 14, Issue 11, Pages 158-164

DOI https://doi.org/10.47310/jpms2025141122

Study of the Effectiveness of Natural Anthocyanin Pigment in Examining Fungal Microscopic Slides

Rabab Majead Abed[1*], Roqia Ahmed Abaas[2] and Fatin Ali Al-Chalabi[3]
[1-3]Department of Biology, College of Education for Pure Sciences, Diyala University, 23001, Iraq

Author Designation: [1]Professor, [2-3]Associate Professor

*Corresponding author: Rabab Majead Abed (e-mail: Rabab.abed@uodiyala.edu.iq).

©2025 the Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0

Abstract The current study was conducted in the Mycology Laboratory of the Department of Life Sciences - College of Education for Pure Sciences during year 2023-2024 with the aim of exploring the possibility of using anthocyanin pigment extracted from some plants in staining fungi. The cold water extract of four plant species was used: leaves of red cabbage (*Brassica oleracea* var. *capitata* f. rubra), tuber of red beet (*Beta vulgaris* L.), seeds of pomegranate (*Punica granatum* L.) and peels of eggplant (*Solanum melongena* L.). The extract was used as a stain in preparing and examining glass slides of two fungi that contaminate stored seeds, which were *Aspergillus niger* and *Rhizopus* sp. and compared with the blue lactophenol stain. The extracts of the four plants showed high efficiency in staining fungi and studying fungal structures clearly, thus outperforming the chemical blue lactophenol. Therefore, this result is important in achieving the Sustainable Development Goals for maintaining health by reducing dependence on chemical compounds and reducing the risks resulting from their continuous use, on the one hand and it is also inexpensive and successful in microscopic studies of fungi.

Key Words Lactophenol, Anthocyanin, Plant, Fungi, *Aspergillus*

INTRODUCTION

Natural pigments have been used for centuries in many industries such as textiles, food, etc. [1]. In recent years, due to the global trend towards sustainable development and environmental conservation, there has been a renewed interest in natural pigments due to their environmental friendliness, availability, low cost, non-toxicity and sustainability [2]. Consequently, natural pigments are rapidly gaining popularity as better alternatives to synthetic pigments [3]. There are many natural sources of pigments, including plants, animals, microorganisms, minerals and some other materials [4]. Plant pigments have a long history of use in various cultures and fields. Plant pigments are extracted from many plants and from different plant parts such as roots, leaves, stems, flowers, fruits and seeds [5]. They provide a wide range of colours. Plant pigments include chlorophyll, which gives the green colour [6]; carotenoids, which are responsible for the yellow and red colours and their shades [7]; anthocyanins, which give colours from orange to dark blue; and Betalains, which give the red or yellow colour [8]. One of the fields in which pigments are used is microscopic preparations, such as the microscopic examination of fungi at different stages of their growth. One of the pigments commonly used in this field is lactophenol blue dye, except that phenol, the main component of the dye, is a carcinogenic and tumour-causing substance that is toxic to humans and the environment [9]. Olise *et al.* [10] and Aqeil and Barhawi [11] investigated the potential of using several plant dyes prevalent in Nigeria to stain several fungal isolates. The results of this study demonstrated the feasibility of using plant dyes in preparing fungal microscope slides [12]. The plants studied included *Garcinia kola*, *Vitex doniana*, *Lantana aculaeta*, *Lawsonia inermis*, *Cnestis ferruginea* and *Pterocarpus soyauxii*. The dye extracted from *P. soyauxii* showed significant potential for staining the fungal isolates [13]:

Therefore, the main aim of the present study is:

- Extracting anthocyanins from different plants
- Testing the ability of extracted from different plants to stain fungal isolates
- Compared to the lactophenol stain (cotton blue)

METHODS

The current study was conducted in the Mycology Laboratory of the Department of Biology, College of Education for Pure Sciences, University of Diyala. The aim was to evaluate the efficacy of some natural stains extracted

from various plants in staining some fungal isolates, in comparison with lactophenol cotton blue, the traditional dye used for staining fungi. The study was conducted during the year 2023-2024 and the steps were as follows:

Preparation of Sabouraud Dextrose Agar (SDA) Medium

The SDA medium was prepared according to the instructions of the medium supplier, Himedia, by dissolving 65 g of SDA medium in 1 litre of distilled water. The medium was stirred using a hot magnetic stirrer and heated to boiling. The pH was adjusted to 5.0-5.5. The medium was then sterilised using an autoclave at 121°C and 1.5 kg/cm^2 for 15 minutes. After the medium cooled, the antibiotic chloramphenicol was added at a rate of 250 mg/L^{-1} to inhibit bacterial growth [14].

Fungi Isolations

To obtain pure isolates of both *Aspergillus niger* and *Rhizopus* sp., five wheat seed samples, each weighing 100 g, were collected from the local market in Baqubah. The samples were transported in plastic bags to the laboratory and fungi were isolated by direct culture on SDA medium, with four seeds per plate. The plates were then placed in an incubator at 27±2°C for seven days or until fungal growth appeared [15]. After fungal growth appeared, a portion of the edge of the fungal colony was taken and transferred to Petri dishes containing SDA medium. The dishes were incubated in an incubator at 27±2°C to obtain pure fungal colonies for later use in staining tests.

Extraction of Anthocyanin Pigments

To extract anthocyanin pigments from various plant sources, red cabbage, beetroot, pomegranate seeds and eggplant peels were used. These plants were obtained from the local market in Baqubah and brought to the laboratory for pigment extraction. The plants were washed with running water, then cut into small pieces. 250 grams of each plant sample was placed separately in an electric mixer. The mixture was filtered after squeezing using Whatman No. 1 filter paper to remove large particles. The filtrate was then taken and heated to boiling for pasteurization. The filtrate from each plant was then stored in opaque glass bottles in a refrigerator at 4°C until use.

Staining of Fungal Isolates

A sample of the fungal colony was taken using a Loop field transfer and placed on a glass slide containing ready-made lactophenol cotton blue stain. The mixture was thoroughly mixed and then fixed by passing the glass slide through a flame several times. The slide was examined under a microscope at 10x and 40x magnification to observe fungal hyphae [16]. Microscope slides of the fungal isolates were also prepared using the stain extract, following the same steps as for the lactophenol blue staining method.

Ethical Approval

This study was conducted in accordance with ethical principles and after obtaining the approval of the Research Ethics Committee in the Department of biology, College of Education for Pure Sciences, University of Diyala, according to document No. 126 dated 10/04/2025.

RESULTS AND DISCUSSION

Staining of the *Aspergillus niger* Fungus

Aspergillus niger fungus belongs to the Ascomycota phylum, whose cell wall contains beta-glucans in addition to chitin. Staining the fungus with anthocyanins extracted from red cabbage (Figure 1) images A and B clearly show the conidia, conidiophore and the bilayered cell wall. The Staining by extracted from red cabbage not clearly show the vesicle. Figure 2 shows the staining of *A. niger* with beetroot extract in both images A and B. We can easily observe the staining of the fungal hyphae and vesicle and conidia were also stained but slightly. It can also be observed that the color was not very dark and the staining was not opaque, which gives greater clarity to the microscope slide. Figure 3 shows the effect of the pigment extracted from Pomegranate seeds on fungal structures. The staining of the fungal sample was light and not opaque and the fungal hyphae, vesicle and conidia can be clearly seen under the microscope. It was found in Figure 4 that all parts of *A. niger* fungus were clearly stained with the extracted from eggplant peel. Lactophenol cotton blue in Figure 5 shows the staining of fungal parts with dye.

Natural pigments demonstrated the same efficiency in highlighting fungal components, such as the cell wall and its layers, conidiophores, conidia and cysts. This result reinforces the possibility of using natural pigments as an alternative to chemical pigments, without the use of any natural dye stabilisers, as is commonly used with chemical pigments. The variation in colour intensity is attributed to the difference in dye concentration in the extract [17,18]. The results of this research are consistent with the study of Olise *et al.* [10], which used natural pigments from some Nigerian plants to dye the yeast Candida albicans and the fungus *Aspergillus niger* the pigments extracted from a number of plants showed their potential for use in dyeing fungi [19].

Staining of the Fungus *Rhizopus* sp.

The fungus *Rhizopus* sp. belongs to the phylum Zygomycota, whose cell wall contains chitosan in addition to chitin. The results of staining with plant pigments, as shown in Figure 6-9, demonstrate a good response of the fungal parts. Figure 6 shows the staining of all fungal structures of the fungus *Rhizopus* sp. with the extracted from red cabbage, photos A and B clearly show the sporangium, spores and sporangiophores, as well as the rhizoids that distinguish this fungus from others. Figure 7-10 also show the staining of all fungal structures of the fungus *Rhizopus* sp. with the extract from red cabbage. Photos A and B clearly show the sporangium, spores and sporangiophores, as well as the rhizoids. Natural dyes demonstrated the same efficiency in highlighting fungal components, such as the cell wall, sporangia and spores, as well as the base of the sporangia, known as rhizoids [20]. This confirms the ability of fungal threads and structures to be dyed with anthocyanin

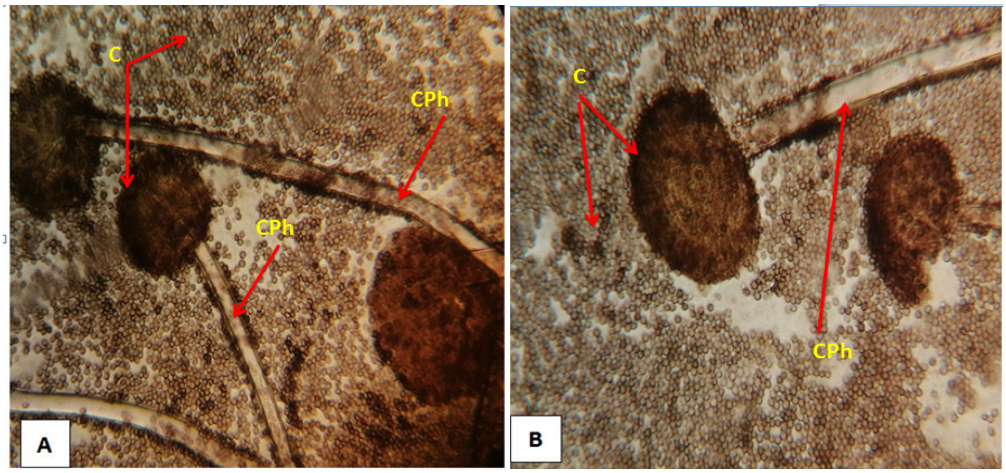

Figure 1(a-b): Staining of the fungus *Aspergillus niger* with anthocyanin pigment extracted from the red cabbage plant, a photo under a light microscope at 40x magnifications
The photos A and B clearly show cph: Conidiophore, c: Conidia

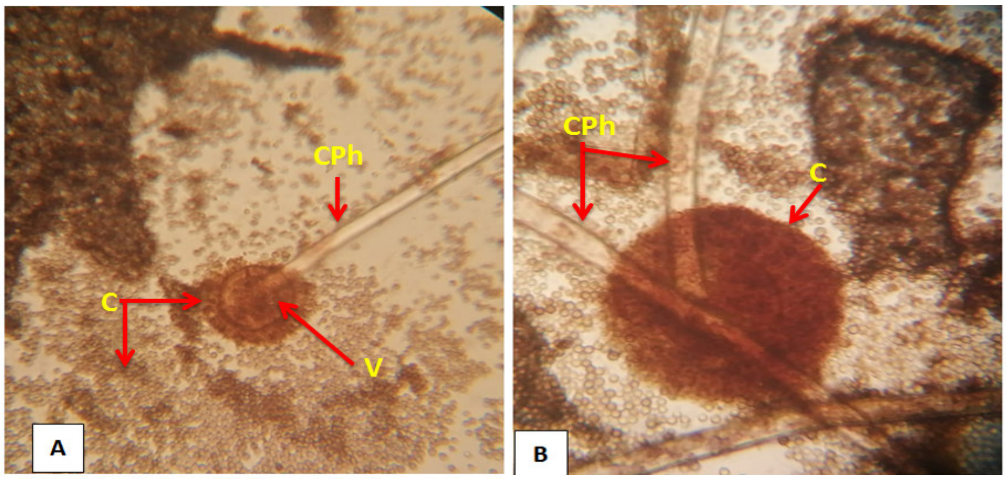

Figure 2(a-b): Staining of the fungus *Aspergillus niger* with anthocyanin pigment extracted from the beetroot plant, a photo under light microscope at a magnification of ×40
The photos A and B clearly show cph: Conidiophore, c: Conidia and v: Vesicle

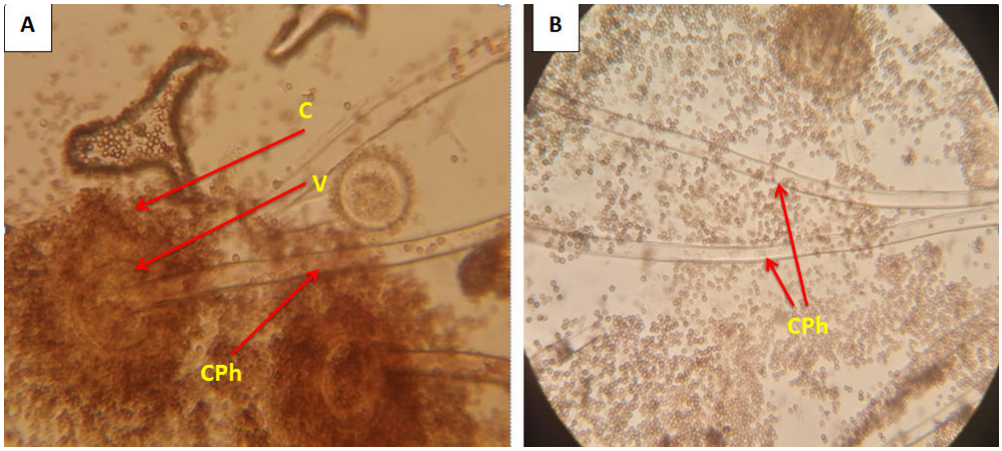

Figure 3(a-b): Staining of the fungus *Aspergillus niger* with anthocyanin pigment extracted from pomegranate seeds, a photo under a light microscope at a magnification of ×40
A and B clearly show cph: Conidiophore, c: Conidia and v: Vesicle

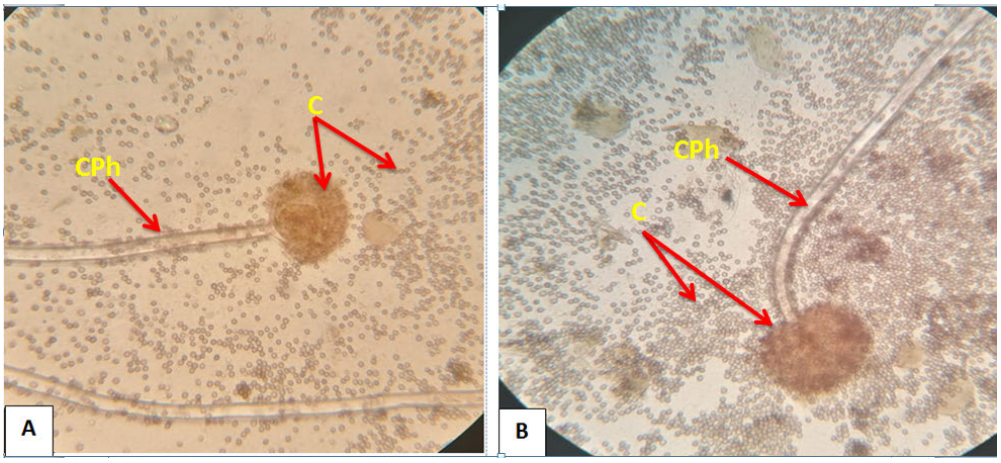

Figure 4(a-b): Staining of the fungus *Aspergillus niger* with anthocyanin pigment extracted from eggplant peels, a photo under a light microscope at a magnification of ×40
A and B clearly show cph: Conidiophore, c: Conidia

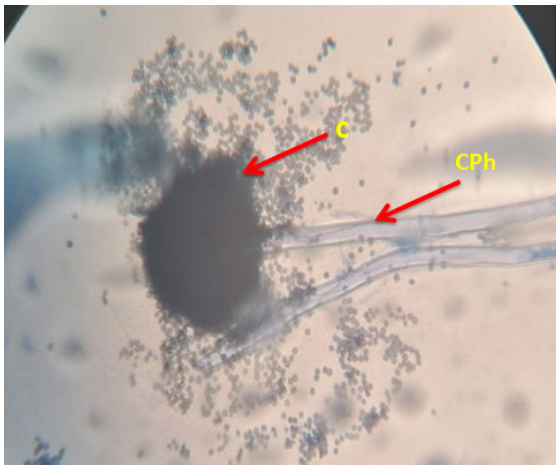

Figure 5: Staining of the fungus *Aspergillus niger* with lactophenol cotton blue stain, a photo under a light microscope at a magnification of ×40
The photo show cph: Conidiophore, c: Conidia

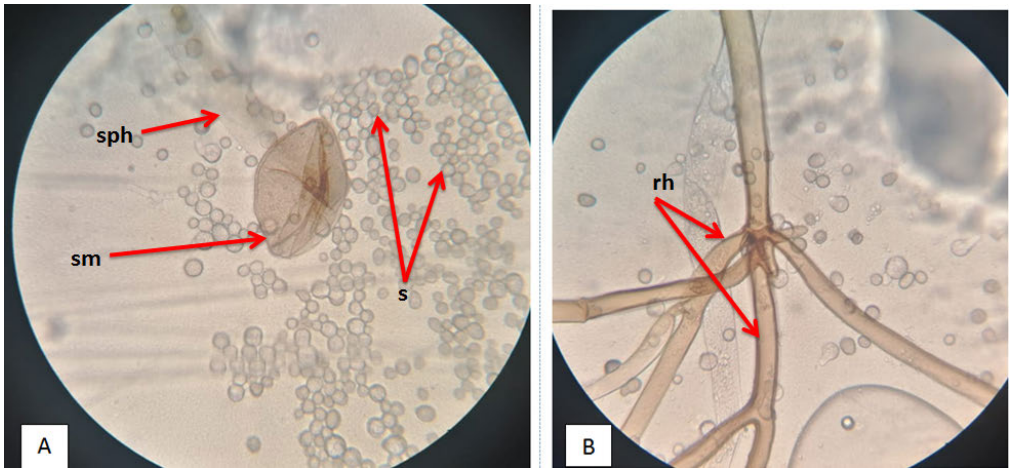

Figure 6(a-b): Pigmentation of the fungus *Rhizopus* sp. with anthocyanin pigment extracted from the red cabbage plant, a photo under a light microscope at a magnification of ×40
Image A showed sm: Sporangium, s: Spores and sph: Sporangiophores, in image B shows rh: Rhizoids

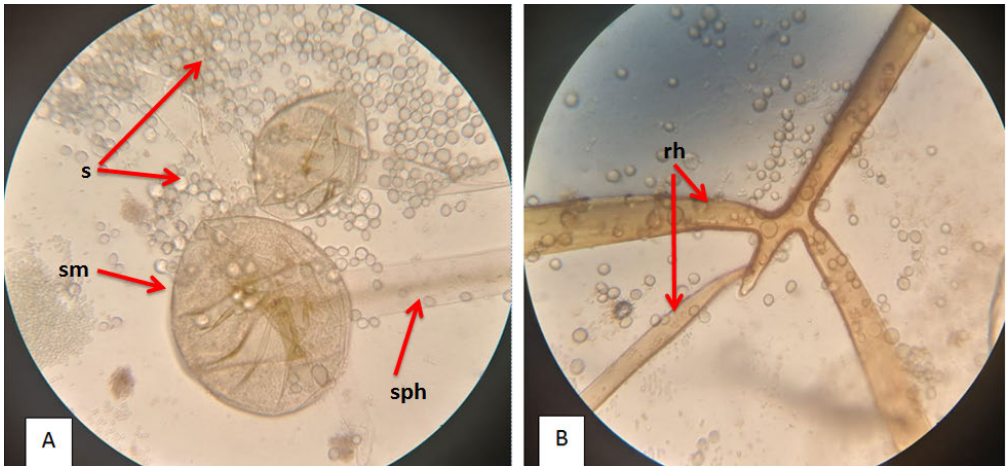

Figure 7(a-b): Pigmentation of the fungus Rhizopus sp. with anthocyanin pigment extracted from the beet plant, a photo under a light microscope at a magnification of ×40
Image A showed sm: Sporangium, s: Spores and sph: Sporangiophores, in image B shows rh: Rhizoids

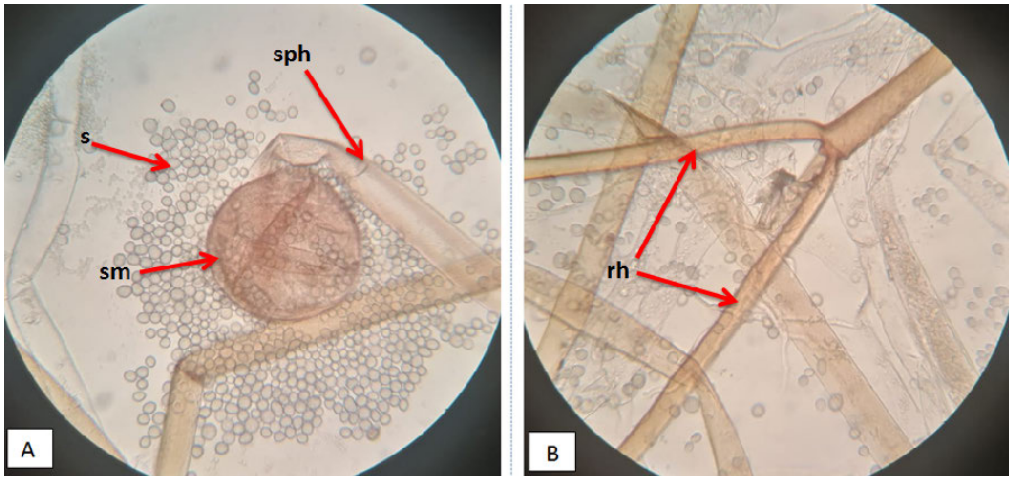

Figure 8(a-b): Pigmentation of the fungus *Rhizopus* sp. with anthocyanin pigment extracted from pomegranate seeds, a photo under a light microscope at a magnification of ×40
Image A showed sm: Sporangium, s: Spores and sph: Sporangiophores, in image B shows rh: Rhizoids

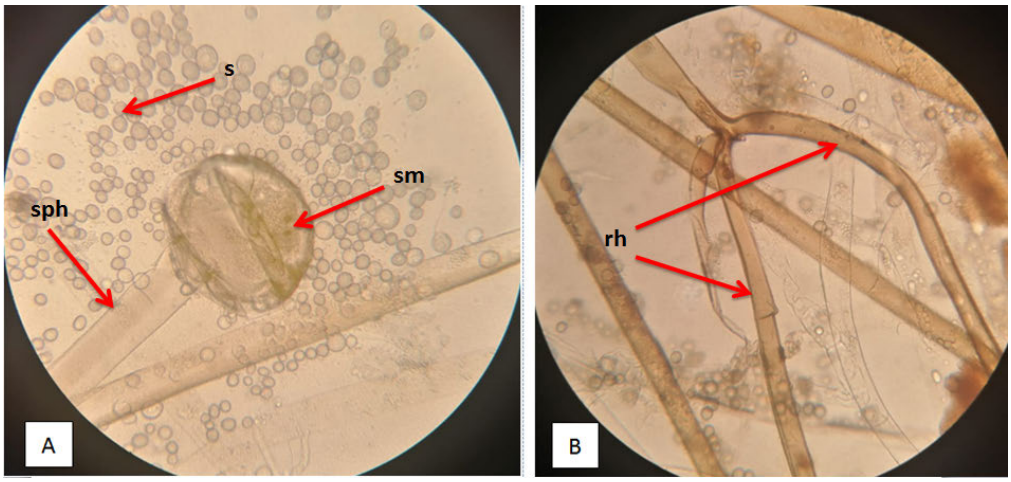

Figure 9(a-b): Pigmentation of the fungus Rhizopus sp. with anthocyanin pigment extracted from eggplant peels, a photo under a light microscope at a magnification of ×40
Image A showed sm: Sporangium, s: Spores and sph: Sporangiophores, in image B shows rh: Rhizoids

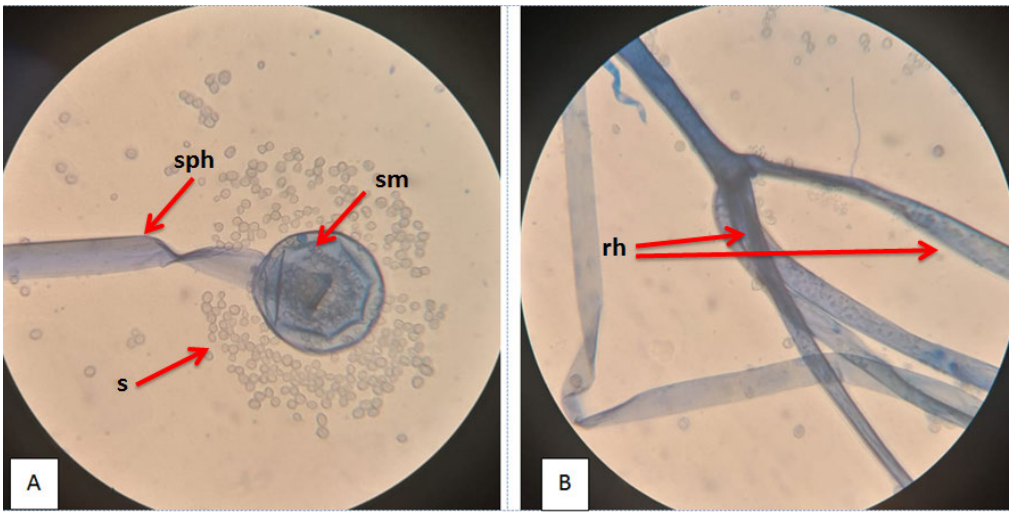

Figure 10(a-b): Staining of the fungus Rhizopus sp. with lactophenol blue stain, a photo under a light microscope at a magnification of ×40
Image A shows sm: Sporangium, s: Spores and sph: Sporangiophores; in image B shows rh: Rhizoids

dye and the ability of the dye to adhere to surfaces without the need for a fixative or mediator between the dye and the tissues to be dyed.

CONCLUSIONS

Natural plant pigments, especially anthocyanins, are easy to extract. Using water for extraction is inexpensive and straightforward compared to other extraction methods and chemical dye preparation techniques. The figures in this manuscript demonstrate the feasibility of using anthocyanins to stain two fungal isolates, one belonging to the Ascomycetes and the other to the Zygomycetes. Despite their differing cell wall compositions, both isolates stained well and clearly with anthocyanins extracted from four different plant sources. This suggests the potential use of these extracts to stain other fungi but not as a replacement for lactophenol blue, as further research on other fungi is needed.

Recommendations

In light of the results obtained by this study, we suggest that the efficacy of plant dyes be tested on a wider range of fungi. Knowledge also recommends determining the chemical properties of plant dyes, as well as testing the shelf life of these natural stains.

REFERENCES

[1] Hsu, W.Y. *et al.* "Molecular Transformations and Self-Association in Anthocyanin Pigment Patterns." *Journal of Biosciences*, vol. 49, 2024, p. 4. https://doi.org/10.1007/s12038-023-00367-x.

[2] Gong, K. *et al.* "Natural Pigment during Flora Leaf Senescence and Its Application in Dyeing and UV Protection Finish of Silk and Wool—A Case Study of *Cinnamomum camphora*." *Dyes* and *Pigments*, vol. 166, 2019, pp. 114-121. https://doi.org/10.1016/j.dyepig.2019.03.037.

[3] Alegba, E.A. and T.O. Uthman. "A Review of History, Properties, Classification, Applications and Challenges of Natural and Synthetic Dyes." *Heliyon*, vol. 10, no. 1, 2024, pp. 1-19. https://doi.org/10.1016/j.heliyon.2024.e33646.

[4] Al-Abdulla, B.Y. and S.D. Oleiwi. "Estimation of Some Phytochemicals in Extract of Eggplant Peels and Cabbage Leaves and Using of Pigment of Them in Drinks Industry." *Mesopotamia Journal of Agriculture*, vol. 47, no. 2, 2019, pp. 128-138.

[5] Melo Miranda, B. *et al.* "Potential of New Plant Sources as Raw Materials for Obtaining Natural Pigments/Dyes." *Agronomy*, vol. 15, no. 2, 2025, p. 405. https://doi.org/10.3390/agronomy15020405.

[6] Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Phenol*. U.S. Department of Health and Human Services, 2008.

[7] Anaam, F.H. *Immunohistological Effects of Gliotoxin Produced by Some Pathogenic Molds* and *Yeasts in Albino Male Mice*. PhD thesis, University of Baghdad, 2016.

[8] Alappat, B. and J. Alappat. "Anthocyanin Pigments: Beyond Aesthetics." *Molecules*, vol. 25, no. 23, 2020, p. 5500. https://doi.org/10.3390/molecules25235500.

[9] Chiegina, N.V. "Mycoflora of Some Salad Vegetables." *Biological Research*, vol. 6, no. 2, 2008, pp. 392-395. https://doi.org/10.3329/ajmbr.v5i4.45266.

[10] Olise, N.A. *et al.* "The Use of Extracts from Nigerian Indigenous Plants as Staining Techniques in Bacteriology, Mycology and Histopathology." *International Journal of Plant Research*, vol. 8, no. 1, 2018, pp. 15-24. https://doi.org/10.5923/j.plant.20180801.03.

[11] Aqeil, A.T. and N.I. Barhawi. "Natural Anthocyanin Dye Is Comparable to Chemical Dyes." *Arabian Journal of Scientific Research*, vol. 1, 2021, pp. 2-7. https://doi.org/10.5339/ajsr.2021.2.

[12] Kakhia, T.I. *Dyes, Colors & Pigments*. 2015. http://tarek.kakhia.org.

[13] Li, G. *et al.* "Research Progress of Betalain in Response to Adverse Stresses and Evolutionary Relationship Compared with Anthocyanin." *Molecules*, vol. 24, no. 17, 2019, p. 3078. https://doi.org/10.3390/molecules24173078.

[14] Pizzicato, B. *et al.* "Advancements in Sustainable Natural Dyes for Textile Applications: A Review." *Molecules*, vol. 28, no. 16, 2023, p. 5954. https://doi.org/10.3390/molecules28165954.

[15] Wang, Z. *et al.* "PgMYB1 Positively Regulates Anthocyanin Accumulation by Activating PgGSTF6 in Pomegranate." *International Journal of Molecular Sciences*, vol. 24, no. 7, 2023, p. 6366. https://doi.org/10.3390/ijms24076366.

[16] Ruta, L.L. and I.C. Farcasanu. "Anthocyanins and Anthocyanin-Derived Products in Yeast-Fermented Beverages." *Antioxidants*, vol. 8, no. 6, 2019, p. 182. https://doi.org/10.3390/antiox8060182.

[17] Verma, S. and G. Gupta. "Natural Dyes and Its Applications: A Brief Review." *International Journal of Research* and *Analytical Reviews*, vol. 4, no. 4, 2017, pp. 57-60.

[18] Li, N. *et al.* "Insight into the Progress on Natural Dyes: Sources, Structural Features, Health Effects, Challenges and Potential." *Molecules*, vol. 27, no. 10, 2022, p. 3291. https://doi.org/10.3390/molecules27103291.

[19] Yungyuen, W. *et al.* "Carotenoid Accumulation and the Expression of Carotenoid Metabolic Genes in Mango during Fruit Development and Ripening." *Applied Sciences*, vol. 11, no. 9, 2021, p. 4249. https://doi.org/10.3390/app11094249.

[20] Zhao, X. *et al.* "Regulation Mechanism of Plant Pigments Biosynthesis: Anthocyanins, Carotenoids and Betalains." *Metabolites*, vol. 12, no. 9, 2022, p. 871. https://doi.org/10.3390/app11094249.